Nosocomial outbreak of *Salmonella* Typhimurium primarily affecting a pediatric ward, South Africa, 2012

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Running head: *Salmonella* Typhimurium outbreak

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ABSTRACT

We describe a nosocomial outbreak of diarrheal disease caused by extended-spectrum β-lactamase producing multidrug-resistant *Salmonella Typhimurium*, focused on a pediatric ward in South Africa. The outbreak peaked between May 2012 to July 2012. Person-to-person transmission was the most likely mechanism of spread of the infection, expedited due to the presence of a breakdown in hand-washing and hygiene, suboptimal infection control practices, overcrowding of hospital wards and an undesirable nurse to patient ratio.

TEXT

*Salmonella* is well recognized as an etiological agent of gastrointestinal and diarrheal disease (1). Outbreaks involving *Salmonella* are well described, with foodborne disease outbreaks very commonly reported (2, 3). To a lesser extent, are the reporting of nosocomial outbreaks of *Salmonella*, with outbreaks occurring among hospitalized humans (4, 5) and outbreaks occurring among hospitalized animals (6). Worldwide, the epidemiology of human *Salmonella* diarrhoeal disease is dominated by only a few non-typhoidal serotypes. In Africa, *Salmonella enterica* serotype Typhimurium (*Salmonella Typhimurium*) and *Salmonella enterica* serotype Enteritidis (*Salmonella Enteritidis*) are the two most commonly reported serotypes of non-typhoidal *Salmonella* (NTS) (7). In the developed world, NTS disease is usually a self-limiting gastroenteritis with low mortality in humans, however in sub-Saharan Africa, NTS also frequently cause invasive disease which is associated with a substantial burden of illness and death (8). In the current study, we report on a nosocomial outbreak of extended-spectrum β-lactamase (ESBL) producing multidrug-resistant *Salmonella Typhimurium* which occurred amongst hospitalized humans in South Africa, 2012.
Names of provinces, districts and hospitals have been concealed to maintain anonymity. Human strains of *Salmonella* Typhimurium were investigated between March 2011 to March 2013, from a particular district (part of a province) of South Africa. Bacterial isolations were performed at a regional clinical microbiology laboratory. The particular district includes several clinics and hospitals which are not within close proximity to a microbiology laboratory, consequently, human specimens are sent for analysis to the regional laboratory. Isolates were then forwarded on the Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases for extended characterization. The CED is the national reference centre in South Africa for human infections due to enteric pathogens including: *Salmonella* species, *Shigella* species, diarrhoeagenic *Escherichia coli* and *Vibrio cholerae*. Isolates from across South Africa are voluntarily submitted to the CED through national laboratory-based surveillance from ~200 clinical microbiology laboratories across the country. As part of routine activities, the CED receives all the above enteric pathogens (surveillance isolates and outbreak isolates) and proceeds to confirm identification, perform serotyping, determine susceptibilities to antimicrobial agents and perform molecular subtyping (if required, as in outbreak situations).

Laboratory methodology included the following. Bacteria were cultured from stool specimens and identified using standard phenotypic microbiological identification and serotyping techniques. Susceptibility testing to antimicrobial agents (ampicillin, ceftriaxone, trimethoprim, sulfamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin, tetracycline and imipenem) was determined by using Etests (bioMérieux, Marcy-l’Étoile, France). The presence of ESBL activity was investigated by combination disk diffusion-based screening tests using ceftazidime (30 µg), cefotaxime (30 µg) and ceftodoxime (30 µg) alone, and in
combination with clavulanic acid (10 µg), as described by the Clinical and Laboratory Standards Institute. Molecular typing of strains was performed using pulsed-field gel electrophoresis (PFGE) analysis, multi-locus sequence typing (MLST) and multiple-locus variable-number tandem-repeats analysis (MLVA). For PFGE, analysis of XbaI digested genomic DNA was performed with a Bio-Rad CHEF-DR III electrophoresis system (Bio-Rad Laboratories, Hercules, USA) using a PulseNet protocol (9). PFGE patterns were analyzed using BioNumerics (version 6.5) Software (Applied Maths, Sint-Martens-Latem, Belgium) with dendrograms of the patterns created using the unweighted pair group method with arithmetic averages, with analysis of banding patterns incorporating the Dice-coefficient at an optimization setting of 1.5% and a position tolerance setting of 1.5%. MLST was performed as per methodology described at the Salmonella MLST database (http://mlst.ucc.ie/mlst/dbs/Senterica), which included DNA sequencing analysis of the following seven housekeeping genes: aroC, dnaN, hemD, hisD, purE, sucA and thrA. DNA sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and an Applied Biosystems 3500 Genetic Analyzer. DNA sequences were collated and analyzed using the DNASTAR Lasergene (version 8.0) Software (DNASTAR, Inc., Madison, WI, USA), followed by analysis at the Salmonella MLST database where allele numbers and a MLST sequence type (ST) were assigned. For MLVA, analysis of repeat DNA sequences at five loci (STTR9, STTR5, STTR6, STTR10 and STTR3) was performed using methodology, nomenclature and reporting of MLVA profiles, according to described methods (10). For MLVA, analysis of DNA fragments was performed using an Applied Biosystems 3500 Genetic Analyzer, followed by data analysis using GeneMapper (version 4.1) Software (Applied Biosystems), followed by analysis at a website, the Institut Pasteur MLVA Database (http://www.pasteur.fr/recherche/genopole/PF8/mlva/) where a MLVA repeat type (RT) was assigned.
In June 2012, routine laboratory-based surveillance detected an unusual increase in the number of human *Salmonella Typhimurium* isolates identified in a particular district of South Africa. By virtue of the sudden increase in number of *Salmonella Typhimurium* isolations, this constituted a potential outbreak. On average, this district usually reports fewer than 5 isolations of *Salmonella Typhimurium* per month, so the sudden identification of more than 5 isolates within a few days raised alarm bells and signaled a potential outbreak. The peak of the outbreak occurred between May 2012 to July 2012 (dates are based on patient specimen collection dates) and further discussion is focused on this peak period. During this peak in the outbreak, 22 cases (patients) were involved. Available information showed that 4 patients were HIV positive, while a further 11 patients had one or more of the following background problems suggesting an immunocompromised state as a potential risk factor for *Salmonella* infection - chronic organ disease, malnutrition, tuberculosis infection or perinatal HIV exposure. Three deaths were recorded; all three of these patients had multiple background medical conditions and *Salmonella Typhimurium* infection was thought to have been a contributory, but not final or singular cause of death. Patients were all hospitalized in a particular hospital; most patients were children between the ages of 1 to 22 months (n=19). For all patients, the median age was 11 months and the interquartile range of age was 15 months. Child patients were from a pediatric ward, while adult patients (n=3) were from multiple wards. Except for an infected adult staff member (discussed later), no apparent links between the other 2 adults and the paediatric ward could be determined. Patients presented with diarrhea and stool specimens were collected for laboratory analysis, with most specimens collected ≥48 hours post hospital admission.
At the regional clinical microbiology laboratory, *Salmonella* species was cultured from stool specimens and so identified as the probable etiological agent of the diarrheal outbreak. Most isolates were then forwarded to the CED for extended characterization. Of the 22 cases which resulted in 32 isolates being identified during the peak of the outbreak, 23/32 (72%) viable isolates were received by the CED - it must be noted that the number of isolates received and analyzed by the CED are often less than that identified at a district/regional laboratory, as some isolates become lost in the system or die in transit. The CED determined that the outbreak strain was multidrug-resistant *Salmonella Typhimurium* (multidrug resistance is defined as resistance to 3 or more classes of antimicrobial agents). The outbreak strain showed resistance to ampicillin, ceftriaxone, trimethoprim, sulfamethoxazole, chloramphenicol and tetracycline; and showing susceptibility to nalidixic acid, ciprofloxacin and imipenem. The outbreak strain also showed ESBL activity. ESBL-producing *Salmonella* are not uncommon in South Africa and these have been previously described, including previous descriptions of ESBL production in *Salmonella Typhimurium* (11, 12). A graph (Fig.1) of numbers of human isolates of *Salmonella Typhimurium* with the identical antimicrobial susceptibility profile (including ESBL production) as the current outbreak strain, for strains isolated in the same district as the outbreak strain, showed a peak of isolates in June 2012, which coincided with the peak of the outbreak. Importantly, note that this graph only illustrates the number of isolates investigated by the CED - we have already explained how the number of strains received and analyzed by the CED is often less than that identified at district level. Molecular typing of the outbreak strains revealed indistinguishable PFGE patterns, indistinguishable MLVA profiles and indistinguishable MLST sequence types. Figure 2 shows the PFGE outbreak pattern, as compared to other/background PFGE patterns identified in the province. The outbreak strain revealed MLVA profile 3-12-10-NA-0211, which was assigned MLVA molecular subtype RTd61 by the Institut Pasteur MLVA
Database. Subtype RTd61 has previously been described in the MLVA database; associated with *Salmonella* Typhimurium isolated in Europe. The outbreak strain revealed MLST molecular subtype ST34. This was not unexpected, as ST34 is a conventional ST and commonly described in *Salmonella* Typhimurium; ST34 and ST19 are the 2 most commonly described STs in *Salmonella* Typhimurium, worldwide including Africa (13, 14). Of note, was that our investigation found no occurrence of ST313; this was also not unexpected, as ST313 is more commonly associated with invasive disease, with reports of this ST313 strain causing epidemic invasive disease in sub-Saharan Africa (15, 16).

Additional molecular typing was performed on randomly selected *Salmonella* Typhimurium isolates from the population in the district served by the regional laboratory associated with the particular hospital in question, with isolation dates preceding and following the peak of the outbreak. For the period March 2011 to April 2012, 28 isolates (~2 for each month) were selected, of which 2 isolates (1 from February 2012 and 1 from April 2012) showed the molecular subtype of the outbreak strain. For the period August to November 2012, 16 isolates (~4 for each month) were selected, of which the majority (10 isolates) showed the molecular subtype of the outbreak strain. This suggests the presence and circulation of the outbreak strain within the community, before and after the peak (May 2012 to July 2012) of the nosocomial outbreak. Therefore, we hypothesis that the outbreak strain was first circulating in the community, prior to its introduction into the pediatric ward of the hospital by a patient(s) or patient contacts. The pediatric ward consists of 25 beds, including 8 beds devoted to patients with gastroenteritis. Further examination of the structural facilities and resource availability revealed several shortcomings which hampered the institution of effective contact precautions and isolation of cases during the outbreak. Firstly and most importantly, inadequate availability of alcohol based hand spray, soap, paper towels and the
lack of a basin in the child toilet during the outbreak period, impaired basic hand hygiene practices. Secondly, although each cubicle has its own hand washing basin, access to the basin was interpreted as inconvenient. Thirdly, small cubicles holding 1 to 6 beds resulted in close physical proximity between patients. Fourthly, it was observed that mothers do not exclusively handle their own children, thus potentially facilitating the transmission of organisms between patients. Finally, an increased influx of patients (June 2012 bed occupancy rate 81% compared to 65% mean for 2012; June 2012 gastroenteritis admissions 61 cases compared to a mean of 32 cases per month for 2012) was observed over the outbreak period. The majority of cases admitted with gastroenteritis during the 3 month period were not thought to be associated with infection by *Salmonella Typhimurium*. It has been suggested (although there is no laboratory evidence to substantiate this), that an initial wave of community acquired, possibly viral gastroenteritis, acted as a precursor event which resulted in increased admissions and pressure on staff, without a concomitant increase in staff numbers. Decompensation in the nursing services would be expected to exacerbate the factors favoring disease transmission within the ward.

Thus, a breakdown in hand-hygiene and infection control practices, secondary to overwhelmed (but also inadequate) ward infrastructural capabilities, combined with an undesirable nurse to patient ratio, then resulted in nosocomial transmission of the strain and amplification of diarrheal cases in the hospital. Available supporting data included the following: 4 cases did not have diarrhea on admission, a further 3 cases had initial cultures on admission which did not yield *Salmonella Typhimurium*, a further 2 patients had a previous (within 12 days) admission to the same paediatric ward, and the time from admission to specimen collection was prolonged with a median time of 4 days (range 1 to 31 days). Also, investigation determined that patients were from diverse geographic regions in the district.
(mostly living in informal settlements) and that there were no common community exposures, such as at children day care centres, common food sources or common social gatherings. For this nosocomial outbreak, food as a source was excluded; it was unlikely that the source of the infection was food prepared by the hospital, because a large majority of patients were infants not consuming hospital food, but rather been fed on ready-made, commercial pre-packed milk feeds which does not require further preparation prior to consumption. In addition, the kitchen prepared and served food to many wards throughout the hospital, yet the outbreak clustered in only a few wards. Testing of a representative sample from the commercially prepared milk feeds failed to culture any pathogens. In terms of identification of a common meeting place in the hospital where patients (and patient contacts) could have gathered together and socialized, no such common area or mechanism was identified. All staff members (n=17) from the pediatric ward were screened for infection by means of testing for culture of pathogens from rectal swabs. *Salmonella Typhimurium* was cultured from 1 staff member, with the isolate showing the same phenotypic and molecular subtyping characteristics as compared to the outbreak strain; further investigation determined that the staff member had a chronic, untreated medical condition and reported chronic diarrhea since 2011. Directional causality relating to the whether the staff member was a recipient of, or participated in the propagation of the outbreak, cannot be determined. Sixteen environmental swabs taken from feeding cups, hand basins, baby cots and resuscitation equipment present in the pediatric ward failed to culture any bacterial pathogens.

The outbreak was eventually contained by institution of the following interventions: firstly, and emphasized as being the most important intervention - through the uninterrupted supply of hand washing consumables and the improvement of hand washing facilities. Secondly, patients and staff were (re)educated on the institution of contact precautions. Thirdly, sick
staff protocols were improved and the infected staff member was counseled. Fourthly, appropriate use of antimicrobial therapy was reinforced. Lastly, the communication of critical microbial results and patterns by the laboratory was enhanced, and the ability of the hospital to mount an appropriate outbreak response was strengthened.

Nosocomial outbreaks of *Salmonella* (including *Salmonella Typhimurium*) are occasionally reported in published literature. In developing countries, nosocomial outbreaks mostly occur in pediatric wards, due to additional risk factors (including malnutrition) associated with NTS infection. Recent reports of nosocomial outbreaks include that of *Salmonella Typhimurium* in a neonatal unit in Turkey (17) and *Salmonella Isangi* in a pediatric ward in South Africa (5).

In Africa, the source and mode of transmission of *Salmonella* is unclear; transmission could include animals, animal products, water and infected humans. Kariuki and coworkers (7) described a population of asymptomatic human carriers of NTS in Kenya and suggested that in Africa, asymptomatic carriers represent an important reservoir of NTS, which may be driving person-person transmission (anthroponotic transmission) of NTS in Africa.

The currently described outbreak investigation has its limitations and shortcomings. Yes, the investigation may not have been conducted in the same systematic way as what an investigation would have been conducted in the USA or Europe. In South Africa, there are acute resource constraints and a shortage of adequately trained personnel to conduct proper outbreak investigations. As a result, most outbreaks are not investigated or inadequately investigated. This is exacerbated by the regular lack of complete data, including the lack of availability of exhaustive clinical data. For the current outbreak, we done our best under the trying circumstances and limited resources available - reporting and publication of some limited data is better than no reporting of data.
In conclusion, due to the limitations described above, a final and unifying theory of the pathogenesis of the outbreak cannot be offered at this time. The outbreak most likely involved person-to-person transmission, expedited due to a breakdown in hand-washing & hygiene, suboptimal infection control practices, overcrowding of hospital wards and an undesirable nurse to patient ratio. Due to the persistence of the immunocompromised niche population of patients within this hospital and indeed other similar hospitals in South Africa, and the transmission dynamics exhibited during the outbreak described, the threat of a similar outbreak persists where factors facilitating the transmission remain.

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REFERENCES


326
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328 **Legend to Figure 1.**
329 Number of human isolates of *Salmonella* Typhimurium with the identical antimicrobial
330 susceptibility profile (including ESBL production) as the current outbreak strain - for isolates
331 from the same district as the outbreak strain, March 2011 to March 2013. Importantly, note
332 that these data only show the number of isolates received and investigated by the CED.
333
334 **Legend to Figure 2.**
335 PFGE patterns (*XbaI* digestion) associated with *Salmonella* Typhimurium strains isolated in
336 the same province in which the nosocomial outbreak occurred. The outbreak pattern is
337 highlighted and so can be compared to other/background patterns identified in the province.
Percentage similarity index

outbreak pattern

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